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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/626,772	07/25/2003	Kazutomo Inoue	0020-5157P	1689

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EXAMINER
SGAGIAS, MAGDALENE K

ART UNIT	PAPER NUMBER
1632	

NOTIFICATION DATE	DELIVERY MODE
08/31/2007	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

mailroom@bskb.com

Office Action Summary	Application No.	Applicant(s)	
	10/626,772	INOUE ET AL.	
	Examiner	Art Unit	
	Magdalene K. Sgagias	1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *20 July 2007*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-11 and 14* is/are pending in the application.

4a) Of the above claim(s) *7,8,11 and 14* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-6, 9-10* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

The finality of the Office action, mailed 1/22/07, is withdrawn. This action is **non-final**.

Applicant's arguments filed 7/20/07 have been fully considered but they are not persuasive. The amendment has been entered. Claims 1-11, 14 are pending. Claims 7-8, 11, 14 are withdrawn. Claims 12-13 have been canceled. Claims 1-6, 9-10 are under consideration.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-6, 9-10 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

The claims are directed to a method for inducing differentiation of mammalian embryonic stem cells into insulin-producing cells, which comprises performing the following steps, in order from (A) to (D) as follows: (A) propagating mammalian embryonic stem cells together with feeder cells with a medium comprising leukemia inhibitory factor; (B) generating embryoid bodies from propagated cells obtained from step (A) by culturing the cells obtained from step (A) in the absence of feeder cells in a medium comprising leukemia inhibitory factor and basic fibroblast growth factor (bFGF) in a suspension culture to give embryoid bodies; (C) the obtained embryoid bodies by incubating the embryoid bodies in a selection-expanding medium; and then (D) differentiating the cells obtained from the step (C) with a differentiation medium to give insulin producing cells.

The specification teaches the expansion of undifferentiated mouse ES cell line 129sv on a feeder layer in the presence of leukemia inhibitory factor (LIF) (**step 1**); formation of embryoid bodies (EBs) in suspension in the presence of LIF and bFGF (**step 2**); selection-expanding of EBs obtained in step 2 on type IV collagen coated dish in medium containing LIF and bFGF used in step 2 and then after culture for 48 hours the medium was replaced with serum free medium supplemented with insulin, progesterone, fibronectin and **nicotinamide** (**step 3**); after 7 days culture in selection-expanding medium **further differentiation** was induced by culturing the cells in serum free medium supplemented with insulin, progesterone, putrescine, laminin and nicotinamide (**step 4**) (see pages 19-21). The specification further discusses that in step 4 the cell clusters can differentiate into either pancreatic islet like cell clusters or nerve like cells (p 15, lines 23-24). The specification however, fails to provide any teachings as to the conditions under which the cells will differentiate into insulin producing cells and prevent them from differentiation into nerve like cells. Thus, as enablement requires the specification to teach how to make and use the claimed invention, the specification fails to enable the claimed methods for inducing differentiation of ES cells into insulin producing cells and not into nerve like cells. It would have required undue experimentation to make and use the claimed invention without a reasonable expectation of success.

In general, the state of the art of directly differentiating ES cells is unpredictable. For example, Verfaillie *et al.* [**Hematology** (Am Soc Hematol Educ Program). 2002;:369-91] review the state of the art of stem cells at the time of filing, teach, that, with regard to the directed differentiation of ES cells, "Many proposed applications of human ES cells are predicated on the assumption that it will be possible to obtain pure populations of differentiated cells from the ES cultures. It might be envisioned that in order to achieve this one would treat ES cells with inducing agents that would convert them with high efficiency to a cell type of interest. In

practice, that has not proven possible [even] with the mouse system." See p. 278, 2nd column, Differentiation in vitro. They further teach that a range of approaches have been attempted to produce a highly homogenous population of differentiated cells from ES cells, for example, relying upon the spontaneous differentiation of the ES cells to embryoid bodies. However, embryoid bodies contain a range of differentiated cells, which is a recognized limitation of directed differentiation of ES cells. Verfaillie teach that the ES cells can be treated with particular agents/factors that can drive differentiation along a specific lineage (see p. 379, 1st column, 1st full ¶. However, it is clear that directed differentiation of ES cells to generate a particular cell type of interest is unpredictable. Thus, specific guidance must be provided to enable the claimed invention.

In the instant invention, the claims require the production of insulin-producing cells. This is found to be unpredictable because the state of the artat the time of filing the art taught that inducing differentiation of insulin producing cells was unpredictable with regard to achieve targeted differentiation of embryonic stem cells into insulin producing cells. **Otonkoski et al,** (Annals of Medicine, 37:513-520, 2005) notes the generation of insulin-producing islet like structures from mouse ES cells it was shown that the generated insulin-expressing cells had neuronal features and cells taken for immature beta-cells might as well be developing neurons (p 517, 1st column, 1st paragraph). Mouse ES cell differentiation towards a beta-cell like phenotype represent an aberrant differentiation pathway of neuroectodermally committed cells, and do not provide a platform for the generation of long-term viable physiologically functioning beta-cells (p 517, 2nd column, 1st paragraph). Otonkoski notes to generate beta-cell is a difficult challenge because the spontaneous differentiation of ES cells in vitro is directed towards neuroendoderm rather than mesoderm or endoderm (p 517, 2nd column, 1st paragraph). Insulin has been detected in differentiating human EBs, but it is not clear if this reflects true pancreatic

differentiation (p 517,2nd column, 1st paragraph). Insulin-producing clusters were also generated from human ES cells by nestin-selection protocol with minor modifications, increasing the insulin content of the clusters but whether these are true immature beta-cells or neuronal cells remains to be shown (p 517, 2nd column, 2nd paragraph). Otonkoski concludes that much controversy remains regarding whether the insulin-producing cells generated in vitro from ES cells are true (immature) beta cells and the challenge of generating physiologically functioning insulin-producing cells from ES cells requires that the differentiating cells follows a pathway resembling that occurring in the developing embryo (p 517, 2nd column last paragraph, p 518, figure 2). **Assady et al,** (Diabetes, 60: 1691-1697, 2001) notes different human ES cell lines did not result in EB formation and this observation may indicate different characteristics of each human ES-derived cell line suggesting the need to examine the in vitro differentiation of each human ES-derived cell line independently or the need to examine clonal human ES cell lines with well-defined differentiated responses to growth factors (p 1694, 2nd column, last paragraph).

Additionally, the claims read upon culturing embryonic stem cells with any type of feeder cells. However, the breadth of the claims is not enabling. For example, the state of the art of culturing human ES cells is such that it is generally supported that **fibroblast** feeder layers provide factors which are required for the maintenance of undifferentiated state. For example, Lim *et al.* [**Proteomics**, 2:1187-1203(2002)] teach the proteome analysis of conditioned medium from mouse embryonic fibroblast feeder layers to characterize the environment that supports the growth of undifferentiated human ES cells, and to identify factors critical for their independent growth. See *Abstract*. Lim state that, "Despite many years of using mouse embryonic fibroblast cells as feeder support of human ES cells, it is still not clear what these cells provide for their clients. The interaction between these two cell types might take place *via* factors secreted into the medium or into extracellular matrix as well as through membrane-

bound proteins.". See p. 1188, 1st ¶. Lim teach that by utilizing proteomic analysis, unexpected results identify many known intracellular proteins, and that further analysis using serum-containing medium in the presence of ES cells, and using other cell types for feeder layers will be required. See p. 1203, 1st ¶, #4. The specification fails to provide any guidance or teachings as to specific factors which would allow hES cells to be propogated in an undifferentiated state under feeder-free conditions, other than using fibroblast-conditioned medium, and the state of the art clearly shows that these factors are yet to be determined. The state of the art is replete with teachings to show that in the absence of feeder cells, ES cells either differentiate or die. See Thomson (PNAS, 92: 7844-7848, August, 1995). In particular, Thomson teach the derivation of a cloned cell line from a rhesus monkey that remains undifferentiated when grown on mouse embryonic fibroblast feeder layers, but differentiate or die in the absence of the fibroblasts (see p. 7844, *Abstract*). Particularly, Thomson *et al.* state that in the absence of the feeder layers, soluble human leukemia inhibitory factor (LIF) fails to prevent the differentiation of the cells, and that the factors that fibroblasts produce to prevent the differentiation of the cells is yet unknown (see p. 7847, 1st column, 2nd paragraph). Thomson *et al.* further state that human inner cell mass-derived cells were cultured in the absence of feeder layers failed to survive beyond 2 passages (see p. 7848, 1st paragraph). Additionally, the working examples in the specification teach using mouse ES cells grown on mouse embryonic fibroblast feeders (MEFs). Thus, the breadth of the claims is not enabling for the use of any type of feeder cell, other than a fibroblast feeder layer, to propagate ES cells.

Although the state of the art is suggesting that inducing differentiation of mammalian ES cells into insulin-producing cells from EBs might be feasible in the future, the instant specification does not provide any relevant teachings, specific guidance, regarding culture

conditions for inducing differentiation of all mammalian species of EBs into insulin-producing cells in step 4 for overcoming the limitations of inducing differentiation of EBs into neural cells as raised by the state of the art and as discussed in the specification of the instant application. In particular, the specification fails to teach directly differentiating ES cells into insulin-producing cells, as required by the claims, in the absence the production of neural cells. The specification provides no specific, enabled use for a heterogeneous population insulin and neural cells, as is produced in the working examples; accordingly, one of skill in the art would have had to practice undue experimentation to determine how to use the heterogeneous mixture of cells that would be produced by the claimed invention. In addition, the specification fails to provide any guidance or teachings as to specific factors which would allow hES cells to be propogated in an undifferentiated state under feeder-free conditions.

Therefore, in view of the quantity of experimentation necessary to determine the parameters listed above for preventing differentiation of EBs in step 4 into neural cells, the lack of direction or guidance provided by the specification for preventing differentiation of EBs in step 4 into neural cells, the unpredictable state of the art with respect to preventing differentiation of EBs in step 4 into neural cells, the undeveloped state of the art pertaining to preventing differentiation of EBs in step 4 into neural cells, and the breadth of the claims directed to all types of ES cells derived from all mammals, it would have required undue experimentation for one skilled in the art to make and/or use the claimed invention.

Applicant's arguments with respect to rejections under 35 USC 102(a) and 102(e) have been considered but are moot in view of the new ground(s) of rejection.

Conclusion

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Magdalene K. Sgagias whose telephone number is (571) 272-3305. The examiner can normally be reached on Monday through Friday from 9:00 am to 5:00 pm. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Peter Paras, Jr., can be reached on (571) 272-4517. The fax phone number for the organization where this application or proceeding is assigned is (703) 872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll free).

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